THE PRODUCTION AND DECTECTION OF SPECIFIC FERMENTS FOR THE TYPHOID-COLI GROUP *

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The theoretical foundation for the specific ferment reaction of Abderhalden, as applied to physiological and pathological organic disturbances, and the methods of testing for the presence of these ferments have been so widely discussed in our medical literature that any consideration of them at this time is unwarranted. Further, any attempt to state the value of the reaction as a diagnostic aid or to solve the mechanism of the reaction, whether it be a true ferment action or otherwise, does not come within the scope of this paper. Without committing myself to any theory regarding the exact kind of bodies involved, I shall retain the nomenclature of Abderhalden and refer to the process as a specific or a non-specific ferment action, as the case may be. I wish to consider only the formation and manifestation of a specifically reacting substance elaborated by the body in response to the parenteral introduction of foreign protein, as in experimentally induced bacterial infection, or in immunization against bacteria.

In all the work previously reported, but few papers[1] have dealt with the application of the Abderhalden technic to bacterial infection where the specific invading organism itself served as fundament in the dialysis procedure. Generally, the subject has been approached from the angle of its significance as an aid to diagnosis, and in such work tissues which contain the causative organisms or have been altered by the infective processes have offered suitable and accessible materials as

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1. Abderhalden and Andryewsky: München. med. Wchnschr., 1913, 60, p. 1641.
Fekete and Gál: Monatschr. f. Geburtsh. u. Gynäk., 1914, 39, p. 21.
Gumpertz: Beitr. z. Klin. d. Tuberk., 1914, 30, p. 201.
Jessen: Ibid., 1913, 28, p. 489.
Kirschbaum and Köhler: Wien. klin. Wchnschr., 1914, 27, p. 837.
Massi: Riv. d'ig. e san. pubb., 1914, 25, p. 295.
Miessner: Deutsch. tierärztl. Wchnschr., 1913, 21, p. 417.
Voelkel: München. med. Wchnschr., 1914, 61, p. 349.

substrates. Particularly in tuberculosis and syphilis has this been true.[2] In the few cases where the organisms, or preparations of the organisms, such as the tuberculins and luetin, have been employed generally good results[3] have been obtained, and the differential reactions obtained between tuberculous tissues and the tuberculins are considered of clinical importance.[4]

With respect to the degree of absolute specificity which exists in ferment production against bacterial fundaments and which may be detected by means of the reaction, but little has been done, and the results are somewhat contradictory. Fekete and Gál[5] reported that, in immunized animals, specificity of ferment production could be detected to the extent that staphylococcus could be differentiated from typhoid and colon bacilli. They were unable, however, to distinguish between typhoid and colon bacilli by means of the reaction. Complete details of their method are lacking in their report.

Kirschbaum and Köhler,[6] using the sera of highly immunized horses, attempted to determine specifically of ferment action for the cholera, typhoid, paratyphoid, and dysentery bacilli. The results were very discordant, a condition which may be explained by the fact that the sera employed were from ten days to two months old.

In the preceding article, in my work with staphylococcus, streptococcus, pneumococcus, M. catarrhalis and B. influenzae, a complete specificity of ferment action was demonstrated. The present paper deals with a natural continuation of that work.

2. Baeslack: Jour. Am. Med. Assn., 1914, 62, p. 1002.
Fränkel: Deutsch. med. Wchnschr., 1914, 40, p. 589.
Gumpertz: Beitr. z. Klin. d. Tuberk., 1914, 30, p. 201.
Gwerder and Melikjanz: München. med. Wchnschr., 1914, 61, p. 980.
Jessen: Beitr. z. Klin. d. Tuberk., 1913, 28, p. 489.
Lampé: Deutsch. med. Wchnschr., 1913, 39, p. 1774.
Melikjanz: Ibid., 1914, 40, p. 1369.
Meyer-Betz: Ibid., p. 826.
Reines: Wein. med. Wchnschr., 1914, 64, p. 368.
Varney and Morse: Jour. Michigan Med. Soc., 1914, 13, p. 515.
Voelkel: Ibid., p. 349.
Wegener: Ibid., p. 15.
3. Abderhalden and Andryewsky: München. med. Wchnschr., 1913, 60, p. 1641.
Fränkel: Deutsch. med. Wchnschr., 1914, 40, p. 589.
Gumpertz: Beitr. z. Klin. d. Tuberk., 1914, 30, p. 201.
Gwerder and Melikjanz: München. med. Wchnschr., 1914, 61, p. 980.
Jessen: Beitr. z. Klin. d. Tuberk., 1913, 28, p. 489.
Krim: Russk. Vrach., 1913, 12, p. 1502.
Lampé: Deutsch. med. Wchnschr., 1913, 39, p. 1774.
Melikjanz: Ibid., 1914, 40, p. 1369.
Meyer-Betz: p. 826.
Reines: Wein. med. Wchnschr., 1914, 64, p. 368.
4. Fränkel: Deutsch. med. Wchnschr., 1914, 40, p. 589.
Gwerder and Melikjanz: München. med. Wchnschr., 1914, 61, p. 980.
Jessen: Beitr. z. Klin. d. Tuberk., 1913, 28, p. 489.
Lampé: Deutsch. med. Wchnschr., 1913, 39, p. 1774.
Wolff and Frank: Berl. klin. Wchnschr., 1914, 51, p. 875.
5. Fekete and Gál: Monatschr. f. Geburtsh. u. Gynäk., 1914, 39, p. 21.
6. Kirschbaum and Köhler: Wien klin. Wchnschr. 1914, 27, p. 837.

To obtain the ferment-containing sera, rabbits were subjected to an immunizing treatment of five injections of B. coli communis, B. coli communior, paratyphoid (A), paratyphoid (B), and typhoids "Hopkins" and "Rawlings." The experiment has been conducted twice, and in both cases duplicate animals were immunized against each organism. Absolute uniformity in results was obtained. It may be said that not all supposedly normal rabbits are suitable for the work, since occasionally the serum of an animal will give positive reactions when combined with any substrate. This source of error was carefully controlled.

In the dialysis procedure only those thimbles which had proven suitable were used. The substrates employed were heavy emulsions of the bacteria. These were rendered free from ninhydrin-reacting substances by repeated boiling and washing. One cubic centimeter of the serum was combined with one cubic centimeter of the various substrates and allowed to dialyze for sixteen hours at 37 C., as in the Abderhalden technic. Sterility was considered essential. Serum and substrate controls were also necessary. At the end of the period of incubation, the dialysates were tested as usual.

The results speak for a high degree of specificity. The sera of rabbits immunized against the bacillus coli communis gave a positive reaction when dialyzed with communis as a substrate and a negative reaction when combined with the other organisms, even with so closely related an organism as the bacillus coli communior. Those sera derived from immunization with the bacillus coli communior degraded communior only. The reactions with the paratyphoids (A) and (B) were also specific, as no group reaction was manifested, nor any sign of interreaction between these sera and the individuals of the coli or typhoid groups. The rabbits which had been immunized with typhoid "Hopkins" furnished sera which did not react with either of the coli strains or with the paratyphoids, but which digested the substrates prepared from typhoids "Hopkins" and "Rawlings." Conversely, the sera of the animals immunized with typhoid "Rawlings" degraded both typhoids "Rawlings" and "Hopkins" but did not attack the others.

In addition, rabbits were subjected to immunizing treatment with a mixture of all of the strains. The results were as expected. Sera from these rabbits were able to effect a decomposition of all of the bacterial substrates. It is thus apparent that, under some conditions at least and within certain limits, a specificity of reaction occurs. Also, it is evident that the limit of specificity was reached with the typhoid sera. This indicates that the chemical composition or molecular configuration of the protein molecules in the two typhoid strains are suffi-

ciently similar to be degraded by one reacting body, and that there exists between the natures of the other bacterial proteins a dissimilarity so great that these proteins are incapable of digestion by one ferment (Table 1).

After determining that a true specificity of ferment production and action exists, experiments were conducted to determine the time relationships involved in the appearance of these ferments when the introduced bacterial protein existed in different physical states and the method of introduction was varied. Accordingly, living, killed, and killed sensitized preparations of typhoid "Rawlings" were administered in single injections of 50,000 millions. The methods of introduction were intravenous, intraperitoneal, and subcutaneous. Previous to the injection, trial bleedings were taken and tested to ensure the suitability of the animal for the experiment. During the period of experimentation all food was denied the animals, as enteral digestion has been cited as a cause for aberrant positive reactions.[7]

Following the introduction of the bacteria, bleedings were taken at intervals and tested according to the previously described method. The sera were always used within twelve hours of withdrawal from the animal as it has been repeatedly reported that a serum more than eighteen hours old is unsuited to the dialysis procedure.[8]

The results here reported are based on several determinations of each method employed, and it may be said that with a given method the time of appearance of the active principle never varied by more than three hours, and this only in the case of the animals injected subcutaneously. In general, the variation was less. In reading the results, all questionable reactions were considered negative and only the first well-defined, blue coloration of the dialysate was regarded as the time of appearance. This may not be the period of greatest intensity, for usually the reactions obtained with successive bleedings became increasingly deep in color up to a maximum. This is illustrated by the accompanying typical protocols:

7. Abderhalden and Lampé: Ztschr. f. physiol. Chem., 1913, 85, p. 136.
Ball: Jour Am. Med. Assn., 1914, 62, p. 599; Ibid., 63, p. 1169.
Grey: Bull. Johns Hopkins Hosp., 1914, 25, p. 117.
Lowy: Jour. Am. Med. Assn., 1914, 62, p. 437.
Scherer: Berl. klin. Wchnschr., 1913, 1, p. 2183.
Schulz: München. med. Wchnschr., 1913, 60, p. 2512.

8. Grey: Bull. Johns Hopkins Hosp., 1914, 25, p. 117.
Lowy: Jour. Am. Med. Assn., 1914, 62, p. 437.
Paine: Boston Med. and Surg. Jour., 1914, 170, p. 303.
Scherer: Berl. klin. Wchnschr., 1913, 1, p. 2183.

RABBIT 9, ♀

Bleeding A (normal)	Serum control, 0
	Typhoid substrate, 0
	Serum + typhoid, 0
Injection of 50 thousand million killed, sensitized typhoid "Rawlings" intraperitoneally.	
Bleeding B (2 hours after injection)	Serum control, 0
	Serum + typhoid, 0
Bleeding C (3 hours after injection)	Serum control, 0
	Serum + typhoid, +
Bleeding D (4 hours after injection)	Serum control, 0
	Serum + typhoid + + +
Bleeding E (5 hours after injection)	Serum control, 0
	Serum + typhoid, + + + +
Bleeding F (6 hours after injection)	Serum control, 0
	Serum + typhoid, + + + +
Bleeding G (30 hours after injection)	Serum control, 0
	Serum + typhoid + + + +

RABBIT 10, ♀

Bleeding A (normal)	Serum control, 0
	Typhoid substrate, 0
	Serum + typhoid, 0
Injection of 50 thousand million killed, sensitized typhoid "Rawlings" subcutaneously.	
Bleeding B (17 hours after injection)	Serum control, 0
	Serum + typhoid, 0
Bleeding C (18 hours after injection)	Serum control, 0
	Serum + typhoid, + +
Bleeding D (19 hours after injection)	Serum control, 0
	Serum + typhoid + + + +
Bleeding E (20 hours after injection)	Serum control, 0
	Serum + typhoid, + + + +
Bleeding F (22 hours after injection)	Serum control, 0
	Serum + typhoid, + + + +
Bleeding G (24 hours after injection)	Serum control, 0
	Serum + typhoid + + + +

DISCUSSION OF RESULTS

When the animals were treated intravenously, ferments appeared following an injection of live organisms in two hours, following an injection of killed, sensitized bacteria in one and one-half hours, and following an injection of killed organisms in three hours.

When treated intraperitoneally, the first appearance of ferments occurred after an injection of live typhoid in six hours, after an injection of killed, sensitized in three hours, and after an injection of killed typhoid in five hours.

When injected subcutaneously, a positive serum was first obtained with live typhoid after twenty-four hours, with killed, sensitized after eighteen hours, and with killed typhoid after thirty-six hours.

From this work it appears that the intravenous method of administration is most rapid in its results, and the subcutaneous gives the slowest response (Chart 1).

TABLE 1

SHOWING RESULTS OF EXPERIMENTS

Serum			Date of Last Injection	Date Serum Was Tested	B. coli communis	B. coli communior	Paraty-phoid (A)	Paraty-phoid (B)	Typhoid "Hopkins"	Typhoid "Rawlings"	None
B. coli communis.....	876	♀	Nov. 20	Dec. 14	++++	0	0	0	0	0	0
	877	♂	Nov. 20	Dec. 14	++++	0	0	0	0	0	0
B. coli communior....	880	♂	Nov. 20	Dec. 14	0	++++	0	0	0	0	0
	881	♀	Nov. 20	Dec. 14	0	++++	0	0	0	0	0
Paratyphoid (A).....	882	♀	Nov. 20	Dec. 14	0	0	+++	0	0	0	0
	883	♀	Nov. 20	Dec. 14	0	0	++++	0	0	0	0
Paratyphoid (B).....	884	♂	Nov. 20	Dec. 14	0	0	0	++++	0	0	0
	885	♂	Nov. 20	Dec. 15	0	0	0	++++	0	0	0
Typhoid "Hopkins"..	886	♀	Nov. 20	Dec. 15	0	0	0	0	++++	++++	0
	887	♂	Nov. 20	Dec. 15	0	0	0	0	++++	+++	0
Typhoid "Rawlings"..	888	♀	Nov. 20	Dec. 15	0	0	0	0	+++	++++	0
	889	♂	Nov. 20	Dec. 15	0	0	0	0	++++	++++	0
Mixture	892	♂	Nov. 20	Dec. 15	++++	++++	+++	++++	++++	++++	0
	893	♀	Nov. 20	Dec. 15	++++	++++	++++	++++	++++	++++	0
Normal	...	..	.....	.....	0	0	0	0	0	0	0
None	...	..	.....	.....	0	0	0	0	0	0	

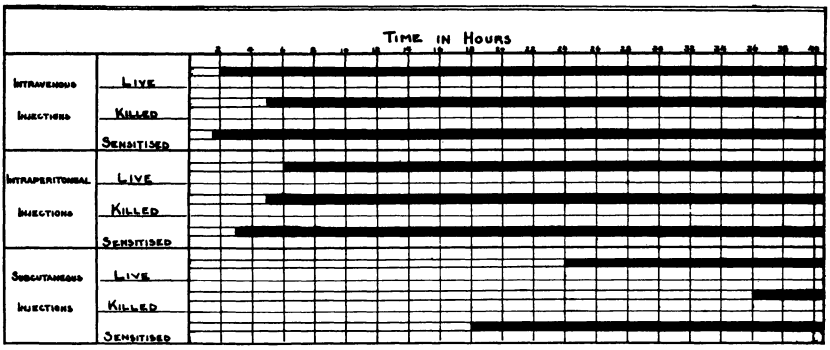

Chart 1.—Showing time relations between intravenous, intraperitoneal and subcutaneous injections.

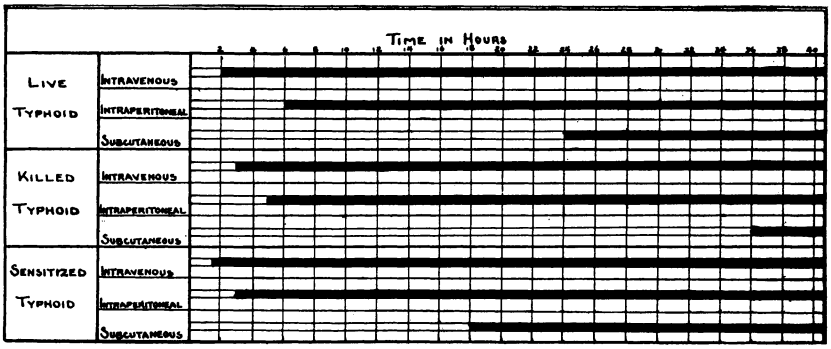

Chart 2.—Showing the relation of kind of material employed to rate of formation of ferments.

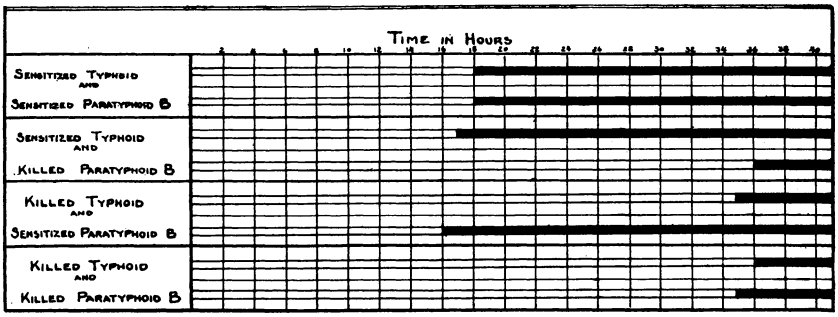

Chart 3.—Showing time relations between simultaneous injections of two kinds of bacilli.

With regard to the type of material employed, the killed, sensitized was most potent in inducing a rapid formation of ferment (Chart 2).

In continuation and substantiation of this conclusion, subcutaneous injections were given simultaneously of killed typhoid and killed paratyphoid (B), of killed typhoid and sensitized paratyphoid (B), of sensitized typhoid and killed paratyphoid (B), and of sensitized typhoid and sensitized paratyphoid (B). When killed organisms of both types were injected, ferments for paratyphoid (B) appeared in thirty-three hours and for typhoid in thirty-six hours. When killed typhoid and sensitized paratyphoid (B) were employed, the serum degraded paratyphoid (B) after sixteen hours and typhoid after thirty-five hours. Reversing the natures of the two types injected, ferments for typhoid were demonstrable after seventeen hours, and for paratyphoid (B) after thirty-six hours. With the final combination in which both types were sensitized, the sera gave positive results with both substrates after eighteen hours (Chart 3).

But one inference can be drawn from this, namely, that the previous treatment of the bacteria with immune serum renders them more susceptible to assimilation by the body, and thus enables them to bring about a more rapid formation of the active principle of parenteral digestion.

CONCLUSIONS

As a result of the parenteral introduction of bacterial protein, some change occurs in the serum of the treated animal, specific ferment formation or otherwise, which causes it to give a positive reaction when combined with its homologous substrate and negative reactions when dialyzed with others.

The method of administration, in a measure, controls the time of appearance of this property; intravenous injections cause the most rapid formation of ferments.

The physical, or chemical state of the material introduced, controls to a degree the time of appearance of this property; the killed, sensitized preparation is most efficient.

The value of the reaction as a means of bacterial differentiation is suggested.

Under proper conditions, the reaction may prove to be an aid to the diagnosis of infectious diseases and may prove of value in indicating the most efficient means to employ in the vaccine therapy of such conditions.